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# Chemical Carcinogens in Bivalve Mollusks from Oregon Estuaries

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# Chemical Carcinogens in Bivalve Mollusks from Oregon Estuaries

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The research undertaken involved the use of indigenous populations of bivalve mollusks as monitors for detecting and quantifying environmental benzo(a)pyrene (BAP) in Oregon estuaries. Short-term and long-term studies were conducted in order to establish baseline levels of BAP and to identify seasonal variations in BAP concentrations in shellfish. A presumptive cellular proliferative disorder, thought possibly to be neoplastic, was also studied in mussels, Mytilus edulis, from Yaquina Bay.

Histological studies revealed that mussels inhabiting polluted environments, and with high BAP body burdens, had an average 6-8% prevalence of the cellular proliferative disorder while those from clean environments and with low or undetectable levels, did not have the disorder. The cellular condition showed a definite seasonal pattern, there was a low prevalence during the summer and fall followed by an increase during the early winter and a peak prevalence occurred in January-February. The atypical, large cells that characterize the disorder in M. edulis possess many ultrastructural properties in common with malignant vertebrate cells.

Further studies are required to evaluate the public health significance of these results. This report was submitted in fulfillment of Contract No. R804427010 by Oregon State University, Corvallis, Oregon.

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# CHEMICAL CARCINOGENS IN BIVALVE MOLLUSKS FROM OREGON ESTUARIES

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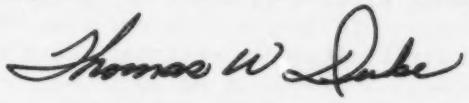
## FOREWORD

The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The EPA Environmental Research Laboratory, Gulf Breeze, contributes to this information through research programs aimed at determining:

- the effects of toxic organic pollutants on individual species and communities of organisms;
- the effects of toxic organics on ecosystem processes and components;
- the significance of chemical carcinogens in the estuarine and marine environments.

Considerable interest has focused recently on the fate and possible effects of carcinogens and mutagens in the aquatic environment which usually is the ultimate receptacle for pollutants. This report describes the fate and some possible long-term effects of polycyclic aromatic hydrocarbons in the marine estuarine environment and biota. These data may serve to alert us to the role of certain carcinogens in the environment generally.



Thomas W. Duke
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## ABSTRACT

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Certain populations of indigenous bivalve mollusks from two industrialized Oregon bays contained shellfish with significant BAP body burdens. Highest levels (>15 ng/g) were detected in mussels inhabiting the Newport bayfront area of Yaquina Bay, and in clams, Mya arenaria, collected near the Coos Bay shipping docks. In general, detectable levels of BAP were found in all populations sampled from the two industrialized bays while populations from non-industrialized bays (Alsea, Netarts) or lightly industrialized bays (Tillamook) contained low or below detectable levels of BAP. BAP body burdens in M. edulis from Yaquina Bay were lowest during the fall, increased during the winter to highest levels in the early spring, after which they declined.

Histological studies revealed that mussels inhabiting polluted environments, and with high BAP body burdens, had an average 6-8% prevalence of the cellular proliferative disorder while those from clean environments and with low or undetectable levels, did not have the disorder. The cellular condition showed a definite seasonal pattern; there was a low prevalence during the summer and fall followed by an increase during the early winter and a peak prevalence occurred in January-February. The atypical, large cells that characterize the disorder in M. edulis possess many ultrastructural properties in common with malignant vertebrate cells.

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## ABBREVIATIONS AND SYMBOLS

### ABBREVIATIONS

| | |
|---|---|
| ANOVA | -- analysis of variance |
| BAP | -- benzo(a)pyrene |
| C | -- celsius |
| $CaCl_2$ | -- calcium chloride |
| DMSO | -- dimethyl sulfoxide |
| EM | -- electron microscope |
| g | -- gram |
| $^3H$-BAP | -- tritiated benzo(a)pyrene |
| hr | -- hour |
| km | -- kilometer |
| KOH | -- potassium hydroxide |
| M | -- molar |
| mg | -- milligram |
| $N_2$ | -- nitrogen |
| ng | -- nanogram |
| PNAH | -- polynuclear aromatic hydrocarbon |
| RUE | -- rate of uptake and elimination |
| TMP | -- 2,2,4-trimethylpentane |
| µm | -- micron |

### SYMBOLS

Oregon Bays

| | |
|---|---|
| A | -- Alsea Bay |
| C | -- Coos Bay |
| N | -- Netarts Bay |
| T | -- Tillamook Bay |
| Y | -- Yaquina Bay |

Bivalve Mollusk Species

| | |
|---|---|
| B | -- *Saxidomus giganteus* (butter or Empire clam) |
| C | -- *Clinocardium nuttalli* (cockle) |
| G | -- *Tresus capax* (gaper clam) |
| M | -- *Mytilus edulis* (blue mussel) |
| O | -- *Crossostrea gigas* (Pacific oyster) |
| S | -- *Mya arenaria* (softshell clam) |

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## SECTION 1

## INTRODUCTION

It has been suggested from analyses of epidemiological data, that 60-90 percent of all cancers occurring in human occupants of industrial societies are caused by environmental carcinogens (e.g. Higgenson, 1971). To date, no definitive data are available on the proportion of human cancers which develop as a consequence of exposure to chemical carcinogens. However, environmental chemicals are suspected to be etiologic agents on the basis of man's proven susceptibility to some chemical carcinogens and the wide occurrence of chemicals which are known to be carcinogenic for vertebrate animals or have structures similar to those of known carcinogens. The effects of chronically exposing indigenous plant and animal species to environmental carcinogens are not known.

Benzo(a)pyrene (BAP) and several other polynuclear aromatic hydrocarbons (PNAH), which are carcinogenic in vertebrate systems, are found in low levels in crude oil, particularly refined oils, and thousands of kg of these compounds enter the sea each year (NAS, 1975). Also, weathered and partially degraded oils may contain additional oxidation products of potential carcinogenicity (Feldman, 1973). However, several recent reports indicate that, in general, PNAH's found at ppb levels in marine animal tissues are derived from common combustion sources and not directly from petroleum contamination (Blumer and Youngblood, 1975; Hites, 1976; Brown and Weiss, 1978). In any event, it seems clear that PNAH levels in economically important marine organisms will continue to increase in biologically productive estuaries because of the inexorable increasing utilization of fossil fuels.

The use of bivalve mollusks for monitoring marine environments in order to detect and quantitate various pollutants, including chemical carcinogens, has been advocated by many investigators (e.g. Goldberg, 1975). Indigenous populations of shellfish seem to be ideal subjects for evaluating carcinogenic PNAH loads in the marine environment (Lee, 1977; Mix et al., 1977a). They are sedentary and, excepting the larval planktonic stage, spend their entire lives in the same location; certain species have cosmopolitan distributions; they inhabit waters that are polluted by all classes of environmental contaminants, including PNAH; they tend to concentrate these contaminants in their tissues; they do not metabolize PNAH; PNAH persist in their tissues after long depuration periods; and they are large, relatively easy to locate, sample, dissect, and prepare for chemical analysis. In addition, bivalve shellfish are widely utilized as a food resource and are exploited by both commercial and recreational fishermen. Thus, qualitative and quantitative analysis of shellfish tissues for specific environmental pollutants constitutes a direct assay method for measuring such contaminants in the marine

environment and offers the potential of using resulting data to assess the public health hazard, if any, of consuming contaminated seafoods.

During the past decade, there have been numerous cases of presumptive sarcomatoid disorders reported in marine bivalve mollusks from areas throughout the world (see review by Mix et al., 1977b). Studies to determine the etiological agent(s) of the putative proliferative disorders have thus far been equivocal. Recently, there have been reports that tentatively linked the appearance of atypical large cells in bivalve populations with oil spills (Barry and Yevich, 1975; Brown et al., 1977; Yevich and Barszcz, 1977), significant body burdens of benzo(a)pyrene (Mix et al., 1977a; in press) and perhaps other aromatic hydrocarbons (Lowe and Moore, 1978), and "adverse water quality" (Alderman et al., 1977; Couch et al., in press). These reports offer some support to the suggestion that general pollution or point-source contamination may be related to increases in neoplasia in marine animals (Kraybill, 1977).

Kraybill (1976) suggested that in order to assess the carcinogenic risk of chemicals as inducers of neoplasms in aquatic organisms, a systematic survey must be made to identify and quantitate carcinogens in waterways, and to determine the body burdens or concentrations in aquatic animals and their relevance to tumor incidence in these species.

The present study was designed to accomplish the following:

1. Evaluate the use of bivalve shellfish as monitors for identifying and quantifying chemical carcinogens in estuaries;

2. Measure baseline levels of BAP in several species of bivalve mollusks from pristine and industrialized Oregon bays;

3. Identify sources of environmental BAP;

4. Evaluate and identify environmental parameters that may influence uptake and elimination and account for any seasonal differences in BAP body burdens;

5. Determine the prevalence of cellular proliferative disorders in mussels (M. edulis) that inhabit uncontaminated waters and environments contaminated with BAP and possibly other PNAH;

6. Determine if there were seasonal differences in the prevalence of the cellular conditions in mussel populations;

7. Describe the ultrastructure of large cells in affected M. edulis and determine whether or not they have features in common with neoplastic, malignant vertebrate cells;

8. To evaluate the public health implications of our findings.

## SECTION 2

## CONCLUSIONS

Utilization of a single species of bivalve mollusk for monitoring an estuary has certain limitations. As a result of these studies, the concept of using multiple species as estuarine monitors for chemical carcinogens has been advanced. There are two primary advantages in using two or more species in monitoring studies. First, it enables investigators to monitor an entire bay, which is not always possible when using a single species. This is particularly important for estuaries that are freshwater dominated. It was found that for Oregon estuaries, softshell clams (Mya arenaria) are useful monitors for upbay sites, where much of the industry is located, since they are virtually the only economically important bivalve mollusk that thrives in these areas of reduced salinity. M. edulis can be used to monitor the lower bays where the salinities are higher. A second advantage is that by using two species which occupy different habitats, it may be possible to obtain information about the routes of PNAH movement (water v. sediments) and reservoirs in the marine environment.

Certain populations of indigenous bivalve mollusks from the industrialized Oregon bays, Yaquina and Coos Bays, contained shellfish with significant BAP body burdens. Highest levels (>15 ng/g) were present in mussels collected from the Newport bayfront in Yaquina Bay and in Coos Bay clams collected near the shipping docks adjacent to Highway 101. In general, detectable levels of BAP were found in all populations sampled from the industrialized bays while populations from non-industrialized bays (Alsea, Netarts) or lightly industrialized bays (Tillamook) contained low or below-detectable levels of BAP. The data suggest that levels of BAP and other PNAH contamination in shellfish are a direct function of the degree of industrialization and/or human activities in the watershed drainages. The sources of BAP and other PNAH in Oregon bivalve mollusks are not precisely known. However, small fuel or oil spills, marinas, industrial wastewater from fish processing and other onshore factories, large ships, creosoted pilings, sewage treatment plants, runoff from storm sewers and watersheds, especially where logging and subsequent slash burning has occurred, and rainout of PNAH produced from combustion during the winter months are all potential sources.

BAP body burdens in M. edulis from Yaquina Bay tended to be lowest during the fall, increased during early winter to the highest levels in early spring, after which they declined. Factors that may be associated with the early spring peaks and perhaps a preceding increase in environmental BAP during the early winter include: reduced water temperatures during the winter; reduced photooxidation of BAP during the winter; resuspension of BAP in the sediments caused by winter floods; rainout of higher levels of atmos-

pheric BAP associated with increased combustion of fuel for heating during the winter; and greatly increased runoff from watersheds during the winter. Intrinsic biological mechanisms may also be involved; changes in BAP body burdens paralleled changes in gonadal maturation during 1977-78.

It should be noted that when BAP body burdens were high, there were also substantial amounts of other fluorescing compounds that, because of the separation techniques utilized, are known to be other PNAH. Thus, BAP essentially serves as an indicator PNAH; the quantitative relationship between BAP concentration and the levels of other PNAH is not yet known.

M. edulis from the moderately polluted Newport bayfront, on Yaquina Bay, contained significant levels of BAP (>15 ng/g), while those from cleaner areas contained very low or undetectable levels of BAP. Histological studies showed that mussels from populations with high BAP body burdens had an average prevalence of 6-8% while those with low or undetectable levels did not have the cellular proliferative disorder. The cellular condition in mussels showed a definite seasonal pattern with a low prevalence during the summer and fall followed by an increase during the early winter and a peak prevalence in January-February. It is not yet known whether or not high body burdens of BAP and other PNAH are directly or indirectly associated with the appearance of the cellular disorder in mussels.

The atypical large cells that characterize the cellular proliferative disorder in M. edulis possess many ultrastructural properties in common with malignant vertebrate cells. The atypical cells have: large, polymorphic nuclei; large, multiple nucleoli; altered Golgi complexes; mitochondrial inclusions; an absence of cytoplasmic differentiation; ribosomes not associated with a membrane system; pleomorphic inclusions in the cytoplasm; and possible asynchronization in the maturation of nucleus and cytoplasm. Nevertheless, alternative explanations concerning their nature can be formulated and much additional information about their life history is required before any definitive conclusions can be made.

Chemical carcinogenesis has recently become a major public health concern. It is known that some chemicals, including BAP and other PNAH, entering the marine environment are either direct carcinogens or carcinogenic if they are metabolically activated by appropriate enzymes. We found detectable levels of BAP in shellfish from 38 of 44 sampling sites, indicating that BAP is rather widespread in Oregon estuaries. Many of the sites were on or near commercial or recreational clam beds. However, a great amount of additional data is required before it is possible to fully assess the public health implications of our findings.

## SECTION 3

## RECOMMENDATIONS

It has been suggested that the presence of neoplastic cellular disorders in feral populations of marine organisms may be indicative of the presence of carcinogens in the environment. It is now established that such disorders occur in Yaquina Bay mussels and our data suggest that such disorders may be linked with environmental contaminants. The implications relative to water quality standards and public health are potentially important. The tentative association between environmental pollution, as indicated by the presence of BAP in biological monitors, and the appearance of proliferative disorders in molluscan populations requires further study. Substantial amounts of additional data are necessary to fully evaluate this potential cause and effect relationship.

Many PNAH, other than BAP, are potent carcinogens. In future studies, efforts should be directed toward: establishing baseline levels of PNAH in monitoring species from pristine and industrialized bays; determining the quantitative relationship between BAP and total PNAH body burdens; identifying specific PNAH present in the tissues of economically important marine organisms; identifying PNAH storage sites in molluscan tissues; and identifying seasonal differences in PNAH body burdens. In order to assess potential public health problems, it is particularly important to expedite the accumulation of this information for marine organisms which are exploited as food sources by recreational and commercial shellfishermen.

An issue that is not yet resolved concerns the exact nature of the large cells that characterize the cellular conditions in bivalve mollusks that have been diagnosed as being neoplastic. The failure to clarify this problem is primarily related to the paucity of information about the complete life history of the cells. In M. edulis, such cells have several cytomorphologic features that are characteristic of malignant vertebrate cells. However, additional studies are required to determine their precise nature.

## SECTION 4

## MATERIALS AND METHODS

### SELECTION OF OREGON ESTUARIES

Oregon has a large number of bays and estuaries (Fig. 1). Three criteria were employed for selecting the 5 estuaries to be studied: they must have major commercial and/or recreational shellfisheries; a variety of bivalve species had to be present in, or on, different substrates; and each bay had to reflect varying degrees of industrialization and human onshore and watershed habitation.

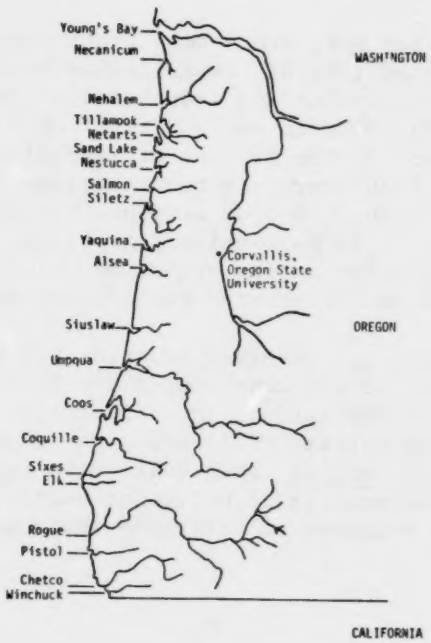


Figure 1. Oregon bays and estuaries.

The following bays were selected for the first studies to determine baseline levels of BAP: Tillamook (Fig. 2); Netarts (Fig. 3); Yaquina (Fig. 4); Alsea (Fig. 5); and Coos (Fig. 6), (accompanying information from Percy, et al., 1974).

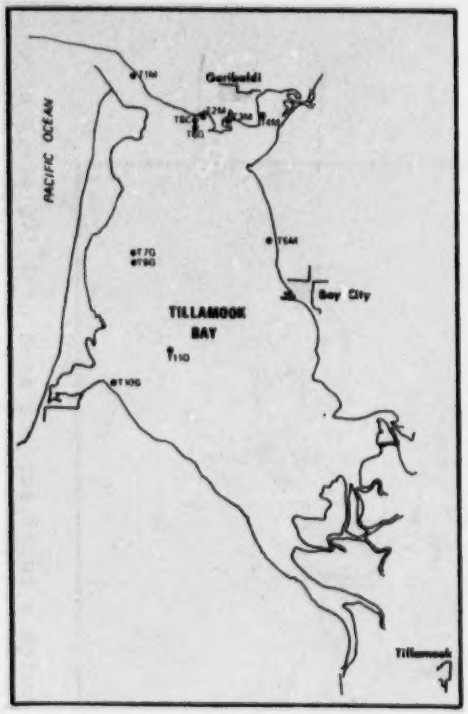


Figure 2. Tillamook Bay, with a total surface area of 8,660 acres of which 50-60% are tidelands, drains a basin of 540 square miles with a high freshwater yield. Major industries: timber, agricultural products, fish and seafoods, tourism. Not considered to be highly industrialized.

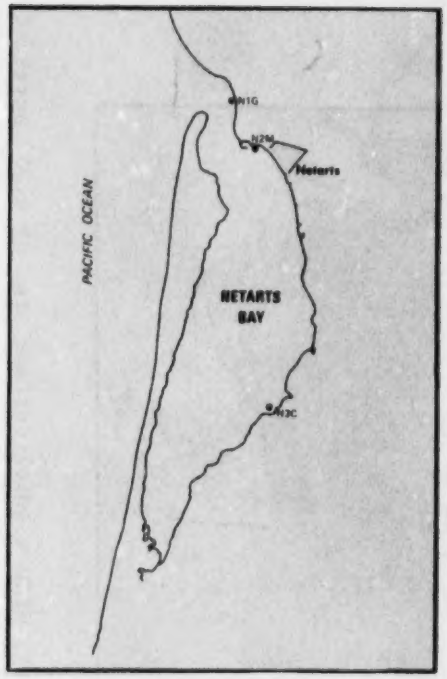


Figure 3. Netarts Bays, with a total surface area of 2,200 acres of which 65-90% are tidelands, drains a basin of 14 square miles with a very low freshwater yield. Manufacturing companies are lacking completely; the bay is considered to be relatively pristine.

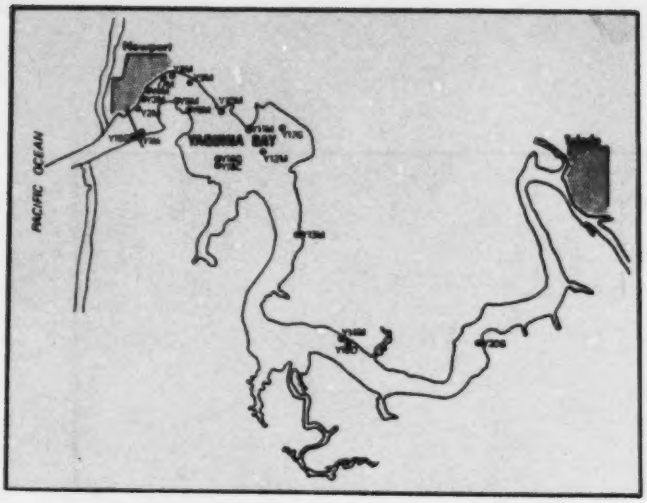


Figure 4. Yaquina Bay, with a total surface area of 4,000 acres of which 35-61% are tidelands, drains a basin of 253 square miles with a medium freshwater yield. A major industrial estuary, the bay is a center for lumbering and commercial fishing activities. Toledo is the focal point of the forest industry processing facilities for the entire Mid-Coast Basin. Newport is the center for commercial fishing activities and there are numerous fish processing plants along the bayfront. Numerous marinas are scattered throughout the bay.

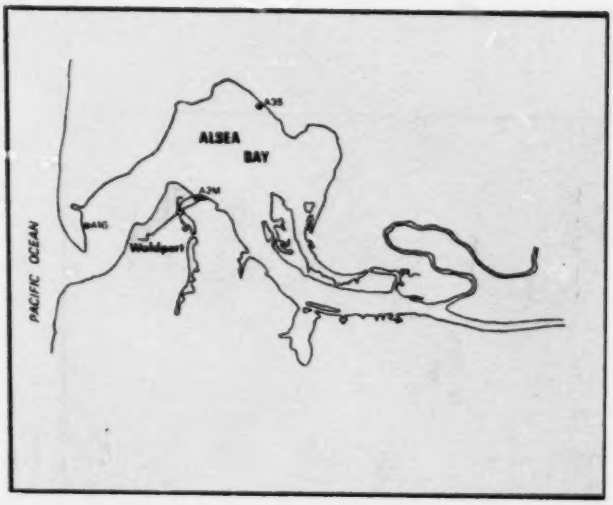


Figure 5. Alsea Bay, with a total surface area of 2,140 acres of which 45-50% are tidelands, drains a basin of 474 square miles with a high freshwater yield. Lumber-related activities, tourism and agriculture are of major economic importance. Little industrial use of the bay.

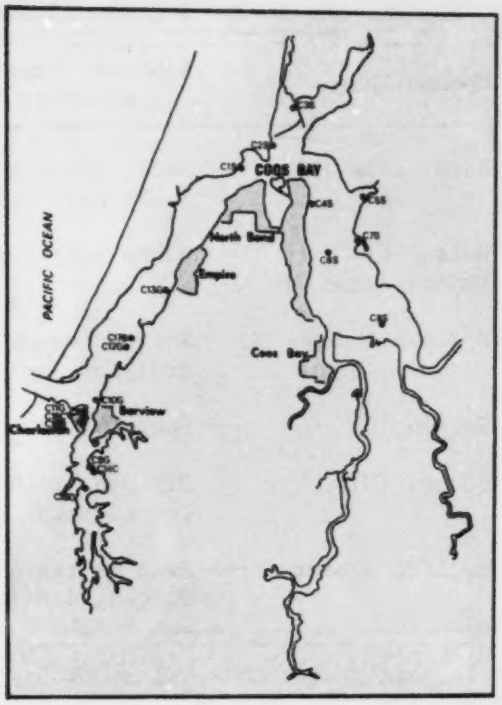


Figure 6. Coos Bay, with a total surface of 10,000 acres of which 50% are tidelands, drains a basin of 605 square miles with a very high freshwater yield. Coos Bay is the most heavily industrialized of all Oregon bays. Timber, fish resources and agricultural activities are of major economic importance. Many major lumber manufacturers are located in the bay and there is heavy shipping traffic concentrated around Coos Bay.

## SELECTION OF BIVALVE MOLLUSCAN SPECIES FOR BASELINE STUDIES

The following criteria were applied for selecting the shellfish species to be analyzed for baseline BAP body burdens. They had to be: economically important (utilized as a food resource by commercial and/or recreational shellfishermen); present in sufficient numbers to withstand sampling for several years; present in at least two of the bays; cosmopolitan, so comparisons could be made with other researchers; representative of certain types of substrates and/or specific habitats (e.g. soft mud, low salinity); available for sampling during the entire year. Table 1 summarizes the species analyzed from the various bays.

TABLE 1. (CLAM, MUSSEL, AND OYSTER SPECIES USED IN THE STUDY)

| Species | Common Name | Habitat, substrate; salinity | Bays |
|---|---|---|---|
| Tresus capax | Gaper clam (G) | Soft, sandy and rocky mud; high | T,N,Y,A,C |
| Saxidomus giganteus | Butter or Empire clams (B) | Rocky mud; high | N,C,Y |
| Mya arenaria | Softshell clam (S) | Soft or sandy mud; low | T,Y,A,C |
| Clinocardium nuttalli | Cockle (C) | Sandy or rock mud | T,N,Y,C |
| Mytilus edulis | Mussel (M) | Pilings, rocks; low to high | T,N,Y,A,C |
| Crassostrea gigas | Pacific oyster (O) | Held in trays, on Sticks, moderate | T,Y,C |

The abbreviations used in this table: T - Tillamook Bay; N - Netarts Bay; Y - Yaquina Bay; A - Alsea Bay; C - Coos Bay.

The abbreviations used to designate the sampling sites (Figs. 2-6) are read as follows: first letter refers to the bay; the number indicates the specific site and the last letter the species. For example, C2S refers to Coos Bay, site 2, softshell clam.

BAYS AND BIVALVE SPECIES UTILIZED FOR LONG-TERM STUDIES

In order to determine temporal or seasonal differences in BAP body burdens, M. arenaria from Coos Bay and M. edulis from Yaquina Bay were studied for two years, from June, 1976 through June, 1978. Mussels and clams from the following sites were assayed for BAP during this period: C2S, C3S, C4S, and C19S; Y1M, Y2M, Y3M, Y4M, Y5M, Y6M, Y7M, Y8M, Y10M, Y11M, Y12M, Y13M, and Y14M.

A brief description of each Yaquina Bay collecting site is included below. Y1M: mussels were collected from a number of weathered, heavily creosoted pilings that formerly supported an old railroad trestle. Y2M, Y3M, Y4M: creosoted pilings that support cold storage facilities and fish processing plants; several nearby marinas. Y5M: creosoted pilings. Y6M: creosoted pilings supporting a small boat dock. Y7M: creosoted floating dock supporting a gas pump; near a large boat basin; Y8M: creosoted floating dock. Y10M: creosoted piling supporting a large ship dock. Y11M: rocks near the liquid natural gas storage tanks. Y12M: creosoted pilings supporting a

marker buoy in the main channel. Y13M: iron pilings near a marina. Y14M: creosoted piling.

## COLLECTION AND PREPARATION OF SHELLFISH SAMPLES FOR CHEMICAL ANALYSIS

Clams and cockles from the various bays were dug during low, usually minus, tides while mussels were collected during the entire tidal cycle, depending on their location. Oysters were obtained from commercial growers and simply removed from the shucking tables. Excepting T. capax, at least 10 shellfish were collected from each sampling site; only 5 of the large gaper clams were sampled. Immediately following collection, the shellfish from a single site were placed in labeled plastic bags, put on ice contained in coolers and transported back to our laboratory in Corvallis. Individual animals were sized, shucked (removed from the shell) and the pooled sample from each site was then weighed. Each pooled sample was then stored in a plastic bag at -20°C until it was analyzed for BAP.

## CHEMICAL ANALYSIS FOR BENZO(a)PYRENE

Prior to analysis, each sample, excepting the mussels, was homogenized in an electric, plastic meat grinder. Mussel samples were prepared similarly; however, because of the small size of the individual animals, no homogenization was necessary. An aliquot of each sample was analyzed according to the method of Dunn (Dunn, 1976). A 30-40 g sample was digested by refluxing in an ethanol-KOH solution. Following digestion, the ethanol-KOH supernatant was extracted with 2,2,4-trimethylpentane (TMP) and the organic phase passed through a column of partially deactivated florisil. The PNAH were eluted with benzene and, after removal of the benzene, the eluate was cleaned up by DMSO extraction in TMP.

BAP was isolated by preparative thin layer chromatography on 20% acetylated cellulose, made to volume in hexadecane and the concentration of BAP determined by spectrophotofluorimetry. Recovery of BAP by the extraction procedure was determined by spiking the original digestion with an aliquot of G-$^{3}$HBAP and counting an aliquot of the final hexadecane solution.

Analysis of wood (piling) samples was accomplished similarly. Approximately 100 mg of the outer 0.2 cm of the piling was dried at 60°C for 24 hr and then placed in a pre-cooled porcelain mortar, liquid $N_2$ added, and the wood pulverized to a fine powder with a porcelain pestle. Ten mg of the powdered wood was then analyzed according to the procedures outlined previously.

## COLLECTION AND PREPARATION OF M. EDULIS FOR HISTOLOGICAL EXAMINATION

After preliminary analyses to measure BAP concentrations in mussels from 14 Yaquina Bay sites, four sites were selected for prevalence studies. Mussels from two of the sites, Y1M and Y12M, initially contained very low or

undetectable levels of BAP (<1 ng/g) while mussels from Y2M and Y4M contained high levels (>15 ng/g). Mussels from all sites were removed from creosoted pilings that had undergone varying degrees of weathering. BAP levels in wood from these pilings were also determined during preliminary studies. The four sites were:

Y1M. This site consists of a number of weathered, heavily creosoted pilings that formerly supported an old railroad trestle. The pilings are located slightly south of the main channel and are subjected to heavy tidal currents.

Y2M and Y4M. Mussels from these sites were removed from creosoted pilings that support two fish processing plants along the bayfront; there are also several marinas nearby. Tidal flows in the bayfront area are minimal.

Y12M. Mussels were removed from creosoted pilings that support a marker buoy in the main channel. This site is also subjected to heavy tidal flows.

Mussels were collected bimonthly from each of the four sites. Immediately after collection, they were separated according to site, placed in labeled plastic bags, put on ice in coolers, and transported to our laboratory in Corvallis. Mussels to be examined histologically were placed in Davidson's fixative (3:3:2:1:1 - 95% ethanol:seawater:formalin:glycerol:acetic acid added just prior to use), processed in the usual way, and sectioned at 6 μm. Tissue slides, prepared for each specimen, were examined microscopically by two or three investigators to determine if they possessed the large cells that characterize cellular proliferative disorders.

## ELECTRON MICROSCOPIC ANALYSIS OF THE LARGE M. EDULIS CELLS

Portions of the visceral mass of living mussels from the Y2M site and several downbay sites were excised and fixed in 0.75% glutaraldehyde, 3% formalin, 0.5% acrolein in 0.1 M sodium cacodylate buffer with 0.02% $CaCl_2 \cdot 2H_2O$, and 5.5% sucrose. Initial fixation was followed by a buffer wash and post-fixation in 1% osmium tetroxide. Tissues were dehydrated, embedded in Spurr medium (Spurr, 1969), and sectioned with either a glass or diamond knife. Thin sections for electron microscopy were triple stained with lead citrate, uranyl acetate, and lead citrate and examined with a Philips EM-301.

## STATISTICAL TREATMENTS

A summary of the statistical tests is included below. A Monroe 1980 Programmable Calculator was used for most of the analyses. A CDC computer was used to determine the quadratic equation from the seasonal prevalence data.

1. Determination of the seasonal-year correlation for BAP body burdens: two way analysis of variance (two way ANOVA).
2. Determination of the seasonal effects on BAP body burdens for a one year period: one way ANOVA.
3. Evaluation of season-BAP body burden correlation: Student Newman-Keuls multiple comparison test.

4. Determination of site-BAP body burden correlation: one way ANOVA.

5. Evaluation of site-BAP body burden correlation: Student Newman-Keuls multiple comparison test.

6. Determination of site-prevalence (cellular proliferative disorder) correlation: one way ANOVA.

7. Evaluation of site-prevalence correlation: Student Newman-Keuls multiple comparison test.

8. Determination of seasonal-prevalence correlation: one way ANOVA.

9. Test seasonal-prevalence correlation to determine if the variation was linear or curvilinear: linear regression analysis.

10. Evaluate seasonal-prevalence correlation: multiple regression analysis.

## SECTION 5

## RESULTS AND DISCUSSION

### BASELINE DATA ON BAP BODY BURDENS

The concentrations of BAP found in bivalves from collecting sites in the 5 bays are included in Table 2. Highest levels (>15 ng/g) of BAP were found in mussels from one site (T3M) in Tillamook Bay (pilings near a gas pump at a marina), and 2 sites (Y2M, Y4M) in Yaquina Bay (pilings beneath fish processing factories and near marinas). Lower levels (>5 ng/g) were found in mussels on pilings near a large ship dock in Yaquina Bay (Y10M) and in softshell clams, also near large ship docks, from Coos Bay (C4S). Shellfish from Netarts and Alsea Bays did not contain detectable levels of BAP.

Although high levels of BAP were found in mussels from Tillamook and Yaquina Bays that were taken from pilings near marinas and fish processing factories, the sources of BAP at these sites have not been clearly established. Because of the suggestion (Dunn and Stich, 1976) that creosote may be the major source of environmental BAP in mussels, the pilings from which they were attached were analyzed from 4 sites. The results are summarized below {site; BAP level in mussels (ng/g); BAP levels in the piling (ng/g)}.

Y1M; 0.22; 265,512
Y2M; 30.30; 137,204
Y3M; 3.25; 6,695
Y4M; 15.00; 107,097

The results of these analyses do not permit the formulation of any firm conclusions. It is interesting to note that the pilings with the highest level of BAP (265,512 ng/g) harbored mussels with one of the lowest levels (0.22 ng/g). However, other factors, such as the age of the piling and proximity to strong tidal currents, must also be considered.

Clam species have not previously been utilized as biomonitors for studying environmental carcinogens. They are buried in the substrate and it may be possible that, because of various physico-chemical processes operating in sediments, food chain decay, utilization of different food sources, or different metabolic capabilities, they will possess lower levels of BAP than mussels even though they inhabit a highly contaminated environment.

The present results suggest that BAP is a ubiquitous contaminant in shellfish inhabiting industrialized Oregon bays. Similar results have also been reported for mussels from Vancouver Harbor (Dunn and Stich, 1975) and

Southern California (Dunn and Young, 1976). With the exception of Coos Bay and portions of Yaquina Bay, no Oregon bay can yet be characterized as being heavily industrialized. However, further industrialization and the concomitant increase in fuel combustion, ship traffic, dredging activities and the number of smaller supporting industries may lead to greater environmental contamination and subsequent increases in PNAH concentrations in economically-important shellfish. Thus, the continuation of monitoring studies in industrialized bays supporting major shellfisheries seems warranted.

TABLE 2. BASELINE LEVELS OF BENZO(a)PYRENE IN BIVALVE MOLLUSKS FROM OREGON BAYS

| Bay site | Species | Type of substrate | Date of collection | B(a)P levels ng/g (ppb) |
|---|---|---|---|---|
| TILLAMOOK | | | | |
| T1M | M. edulis | Rock | 6-29-76 | * |
| T2M | M. edulis | Rock | 6-30-76 | 0.40 |
| T3M | M. edulis | Dock | 6-28-76 | 16.86 |
| T4M | M. edulis | Log | 6-28-76 | 0.28 |
| T5M | M. edulis | Concrete | 6-30-76 | 0.10 |
| T6G | T. capax | Gravel | 6-30-76 | 0.16 |
| T7G | T. capax | Sandy mud | 6-10-76 | 3.21 |
| T10S | M. arenaria | Soft mud | 6-10-76 | 0.30 |
| T11O | C. gigas | Mud | 6-29-76 | * |
| NETARTS | | | | |
| N1G | T. capax | Rocky Gravel | 6-29-76 | * |
| YAQUINA | | | | |
| Y1M # | M. edulis | Piling | 6-15-76 | 0.12 |
| Y2M # | M. edulis | Piling | 6-15-76 | 30.20 |
| Y3M # | M. edulis | Piling | 6-15-76 | 3.15 |
| Y4M # | M. edulis | Piling | 6-15-76 | 14.90 |
| Y5M # | M. edulis | Piling | 6-15-76 | 0.87 |
| Y6M # | M. edulis | Dock | 6-15-76 | 3.00 |
| Y7M # | M. edulis | Dock | 6-15-76 | 4.13 |
| Y8M | M. edulis | Dock | 6-15-76 | 0.39 |
| Y9M | M. edulis | Piling | 6-15-76 | 0.77 |
| Y10M # | M. edulis | Piling | 6-15-76 | 5.23 |
| Y11M # | M. edulis | Rocks | 6-15-76 | 0.51 |
| Y12M # | M. edulis | Piling | 6-16-76 | 0.44 |
| Y13M # | M. edulis | Iron piling | 6-16-76 | 0.37 |
| Y14M # | M. edulis | Dock | 6-17-76 | 4.30 |
| Y15G | T. capax | Sand | 6-15-76 | 0.56 |
| Y16G | T. capax | Sandy mud | 6-15-76 | 0.25 |
| Y17G | T. capax | Soft mud | 6-15-76 | 0.25 |

TABLE 2. Continued

| Bay site | Species | Type of substrate | Date of collection | B(a)P levels ng/g (ppb) |
|---|---|---|---|---|
| ALSEA | | | | |
| A1G | T. capax | Rocky sand | 7-09-76 | * |
| A2M | M. edulis | Dock | 7-09-76 | * |
| A3S | M. arenaria | Soft mud | 7-09-76 | * |
| COOS | | | | |
| C1S | M. arenaria | Sandy mud | 6-28-76 | 0.57 |
| C2S # | M. arenaria | Sandy mud | 6-28-76 | 0.33 |
| C3S # | M. arenaria | Soft mud | 6-28-76 | -- |
| C4S # | M. arenaria | Rocky mud | 7-15-76 | 6.66 |
| C5S | M. arenaria | Soft mud | 7-20-76 | 0.33 |
| C6S | M. arenaria | Sandy mud | 7-15-76 | 0.56 |
| C7S | M. arenaria | Soft mud | 7-28-76 | 0.58 |
| C8S | M. arenaria | Soft mud | 7-15-76 | 1.91 |
| C9G | capax | Sandy mud | 7-29-76 | 0.51 |
| C10G | capax | Soft mud | 6-29-76 | 0.44 |
| C11G | . capax | Sandy mud | 7-29-76 | 0.33 |
| C12G | T. capax | Sandy mud | 6-29-76 | 0.14 |
| C13G | T. capax | Sandy mud | 6-29-76 | 0.35 |
| C16B | S. giganteus | Sandy mud | 7-29-76 | 1.05 |
| C17B | S. giganteus | Sandy mud | 7-25-76 | 0.29 |
| C19S # | M. arenaria | Soft mud | -- | -- |

* Not detectable, less than 0.1 ppb
\# Analyzed bimonthly for two years
-- Not analyzed

LONG-TERM STUDIES ON BAP BODY BURDENS

To evaluate the seasonal influence and temporal fluctuations in BAP body burdens, M. arenaria from 4 Coos bay sites and M. edulis from 13 Yaquina Bay sites were assayed for two years (Tables 3 and 4).

M. arenaria from Coos Bay did not show any consistent patterns of seasonal maximums or minimums. For 1976-77, BAP body burdens were lower in the fall and higher in the spring; in 1977-78, that trend was reversed. None of the bimonthly deviations were statistically significant. Clams sampled from the industrial bayfronts of Coos Bay (C19S) and North Bend (C4S) had greater BAP concentration than those sampled from non-industrialized areas (C2S, C3S) during the two year period.

BAP concentrations varied considerably in mussels from different sites and during different times of the year. Mussels collected along the Oldtown bayfront (Y2M, Y3M, Y4M) generally had the highest BAP concentrations through-

out the two year period. The reasons for this are not known nor are the sources of BAP. It is assumed that these mussels inhabit a more contaminated environment because of their proximity to creosoted pilings, marina, boats and fish-processing plants. Local sources of BAP may include creosote, fuel from boats and marinas, wastewater from fish-processing plants, and/or run-off after periods of heavy rainfall.

There were occasional periods when mussels from certain sites, typically with low BAP concentrations, appeared with unusually high body burdens (e.g. Y6M on 6/29/77). Although analytical error or inadvertent contamination during processing cannot be ruled out, this seems unlikely since other samples, handled in precisely the same manner, did not show such deviations. Small gas or oil spills may have been associated with the sporadic high concentrations.

The data on BAP body burdens in M. edulis from Yaquina Bay were subjected to extensive statistical analyses. To determine if there were significant differences in BAP concentrations in mussels from Y1M-Y14M over the two-year study period, the data were analyzed using a one-way ANOVA. It was found that there were significant differences between sites ($F=8.15>F.01\ [13,137]=1.80$). A Student Newman-Keuls Multiple Range Test showed that only site Y2M differed significantly from the others ($F=10.55>F.01\ [13,137]=2.26$). Mussels from Y2M had significantly higher BAP body burdens than mussels from other sites during the two-year sampling period.

TABLE 3. BAP BODY BURDENS (ng/g) IN M. ARENARIA FROM COOS BAY, OREGON

| Date | SITE | | | |
|---|---|---|---|---|
| | C2S | C3S | C4S | C19S |
| 6/28/76 | 0.33 | 5.56 | 6.66 | ++ |
| 10/20/76 | 0.85 | 0.38 | 3.63 | 12.71 |
| 12/17/76 | 1.27 | 0.42 | 5.31 | 18.32 |
| 2/14/77 | 1.06 | 0.50 | 9.57 | 22.52 |
| 4/08/77 | 3.58 | 0.69 | 8.51 | 31.40 |
| 6/17/77 | 1.43 | 0.53 | 4.17 | 32.54 |
| 8/12/77 | 0.86 | 0.00 | 4.86 | 25.68 |
| 10/14/77 | 1.89 | 1.43 | 12.08 | 7.63 |
| 12/09/77 | 2.96 | 0.28 | 6.24 | 24.98 |
| 2/06/78 | ++ | 0.63 | 8.11 | 17.17 |
| 3/30/78 | 0.54 | 0.07 | 2.88 | 13.08 |
| 6/24/78 | 2.75 | 0.82 | 3.31 | 13.92 |

++ Not sampled

TABLE 4. BAP BODY BURDENS (ng/g) IN M. EDULIS FROM YAQUINA BAY, OREGON

| Date | SITE | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Y1M | Y2M | Y3M | Y4M | Y5M | Y6M | Y7M | Y8M | Y10M | Y11M | Y12M | Y13M | Y14M |
| 6/15/76 | 0.12 | 30.10 | 8.15 | 15.00 | 0.87 | 3.01 | 4.13 | 0.39 | 5.23 | 0.51 | 0.44 | 0.37 | 4.29* |
| 7/22/76 | 4.72 | 67.88 | 4.50 | 6.73 | 4.37 | 2.26 | 2.37 | 0.82 | 10.70 | 0.65 | 0.70 | 0.33 | 0.53 |
| 9/24/76 | 0.68 | 33.84 | 15.74 | 6.88 | 1.15 | 1.90 | 14.27 | 0.86 | 6.30 | NS+ | 0.82 | 0.80 | 0.26 |
| 11/16/76 | 0.62 | 40.20 | 8.46 | 8.95 | 2.71 | 17.39 | 19.07 | NS | 3.60 | 0.45 | 0.45 | 0.90 | 0.57 |
| 12/16/76 | 8.41* | 12.23 | 7.27 | 7.47 | 0.61 | 6.07 | 3.81 | NS | 2.80 | NS | 0.33 | 0.06 | 0.36 |
| 2/03/77 | 3.77 | 32.97 | 71.93* | 170.13* | 0.93 | 8.08 | 1.71 | 1.97 | 3.04 | 0.74 | 0.46 | 0.20 | 0.18 |
| 4/08/77 | 1.72 | 21.89 | 7.91 | 12.67 | 1.48 | 4.44 | NS | NS | 3.77 | 0.69 | 0.50 | NS | 0.38 |
| 6/29/77 | 6.32 | 15.39 | 3.54 | 5.43 | 2.14 | 50.52* | 3.51 | 1.46 | NS | 1.96 | 0.36 | 0.00 | 0.35 |
| 8/29/77 | 1.19 | 5.14 | 2.82 | 2.24 | 5.64 | 4.42 | 5.78 | NS | NS | 0.41 | 0.00 | 2.57 | 0.33 |
| 10/13/77 | 0.82 | 4.97 | 5.35 | 1.86 | 1.22 | 3.16 | 4.24 | 0.52 | 4.19 | 0.13 | 0.24 | 0.37 | 0.34 |
| 12/08/77 | 1.24 | 15.68 | NS | 6.38 | 4.73 | 3.07 | 36.99 | 8.14* | 9.42 | NS | 0.00 | NS | NS |
| 2/03/78 | 3.12 | 27.73 | NS | 13.72 | 7.72 | 32.59 | NS | 2.34 | 10.59 | 2.98 | 1.18 | NS | NS |
| 4/28/78 | 0.70 | 20.70 | NS | 5.52 | 1.16 | 4.04 | 27.42 | 2.68 | NS | NS | 0.10 | NS | NS |
| 6/24/78 | 0.83 | 29.27 | NS | 17.50 | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| AVERAGE BODY BURDEN | 1.99 | 25.57 | 6.53 | 8.49 | 2.67 | 7.54 | 11.21 | 1.38 | 5.96 | 0.95 | 0.40 | 0.7 | 0.37 |

* Data not included in statistical analyses.
+NS Not sampled or not yet analyzed.

Each piece of data from Table 3 was then transformed into a "percentage of the mean" in order to obtain a universal unit that could be used in analyzing the data. For example, the average body burden of BAP in mussels from Y1M and Y2M during the two years was 1.99 and 25.57 ng/g, respectively. The fact that on 4/8/77, mussels from Y1M contained 1.72 ng/g BAP while those from Y2M had 21.89 ng/g means only that those from Y2M were more contaminated; such data alone could not be used to measure seasonal differences. However, 1.72 ng/g is 86.43% of the mean (1.99 ng/g) body burden in mussels from Y1M or -13.57% of the mean; similarly, mussels from Y2M had a -14.39% of the mean body burden. These percentages of the mean could then be used to evaluate seasonal differences. Table 5 contains the transformed data.

Figure 7 portrays graphically the data included in Table 5. There were no obvious deviations during 1976-77 while there was a noticeable increase in BAP body burdens during the late winter and early spring of 1977-78.

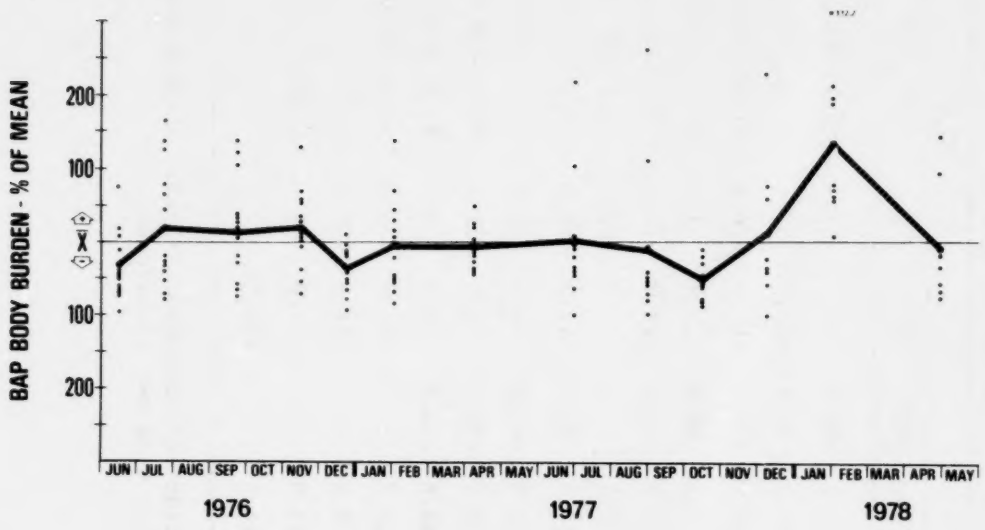


FIGURE 7. Seasonal differences in BAP body burdens in M. edulis populations from Yaquina Bay, Oregon. Each dot represents the BAP body burden, expressed as a percentage of the mean during the two-year period, from a single site. Each open circle is the mean of the data points for each sampling period (e.g., June 1976, October 1977).

TABLE 5. BAP BODY BURDENS CALCULATED AS A PERCENTAGE OF THE MEAN

| Date | Day of the year | SITE | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Y1M | Y2M | Y3M | Y4M | Y5M | Y6M | Y7M | Y8M | Y10M | Y11M | Y12M | Y13M | Y14M |
| 6/15/76 | 167 | -94.0 | 17.7 | -51.8 | 76.7 | -67.4 | -60.1 | -63.2 | -71.7 | -12.2 | -46.3 | 10.0 | -47.1 | |
| 7/22/76 | 204 | 137.2 | 167.5 | -31.1 | -20.7 | 63.7 | 70.0 | -78.9 | -40.6 | 79.5 | -31.6 | 75.0 | -52.9 | 43.2 |
| 9/24/76 | 268 | -65.8 | 32.3 | 138.0 | -19.0 | -56.9 | -74.8 | -27.3 | -37.7 | 5.7 | | 105.0 | 14.3 | -29.7 |
| 11/16/76 | 321 | -68.8 | 57.2 | 29.6 | 5.4 | 1.5 | 130.6 | 70.1 | | -39.6 | -52.6 | 12.5 | 28.6 | 54.0 |
| 12/16/76 | 352 | * | -52.2 | 11.3 | -12.0 | -77.2 | -19.5 | -66.0 | | -53.0 | | -17.5 | -91.4 | -2.7 |
| 2/03/77 | 44 | 69.4 | 28.9 | | | -65.2 | 7.2 | -84.8 | 42.8 | -49.0 | -22.1 | 15.0 | -71.4 | -51.4 |
| 4/08077 | 98 | -13.6 | -14.4 | 21.1 | 49.2 | -45.6 | -41.1 | | | -36.7 | -27.4 | 25.0 | | 2.7 |
| 6/29/77 | 180 | 217.6 | -39.8 | -45.8 | -38.4 | -19.8 | | -68.7 | 5.8 | | 106.3 | -10.0 | -100.0 | -5.4 |
| 8/29/77 | 241 | -40.2 | -79.9 | -56.8 | -73.6 | 111.2 | -41.4 | -48.4 | | | -56.8 | -100.0 | 267.1 | -10.8 |
| 10/13/77 | 286 | -58.8 | -80.6 | -18.1 | -79.1 | -54.3 | -58.1 | -62.2 | -62.3 | -29.7 | -86.3 | -40.0 | -47.1 | -8.11 |
| 12/08/77 | 342 | -37.7 | -38.7 | | -24.8 | 77.2 | -59.3 | 230.0 | | 58.0 | | -100.0 | | |
| 2/03/78 | 34 | 56.8 | 8.4 | | 61.6 | 189.1 | 332.2 | | 69.6 | 77.7 | 213.6 | 195.0 | | |
| 4/28/78 | 87 | -64.8 | -19.0 | | -35.0 | -56.6 | -46.4 | 144.6 | 94.2 | | | -75.0 | | |
| 6/24/78 | 175 | -58.3 | 14.5 | | 106.1 | | | | | | | -100.0 | | |

* Blanks indicate that either no data were available because the site was not sampled or the samples were not analyzed, or the data were not transformed.

There were dramatic differences in weather during the two-year study; the 5-month period from October, 1976 - February, 1977 was the driest ever recorded, while the same period for 1977-1978 was generally normal. Figures 8 and 9 contain information for 1976-77, 1977-78, on the amount of rainfall on the Yaquina Bay watershed and the temperature and salinity of the estuary where the mussels were sampled.

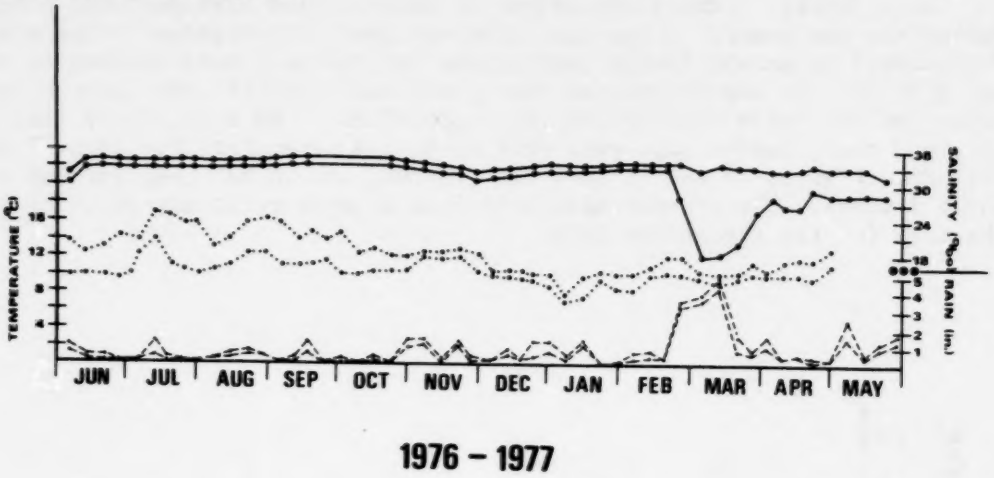


Figure 8. The amount of rainfall (broken line) in the Yaquina Bay watershed (each point represents 1 week) and the temperature (dotted line) and salinity (solid line) of Yaquina Bay adjacent to the O.S.U. Marine Science Center during 1976-77. Each point for the salinity and temperature profiles represents the weekly maximum and minimum.

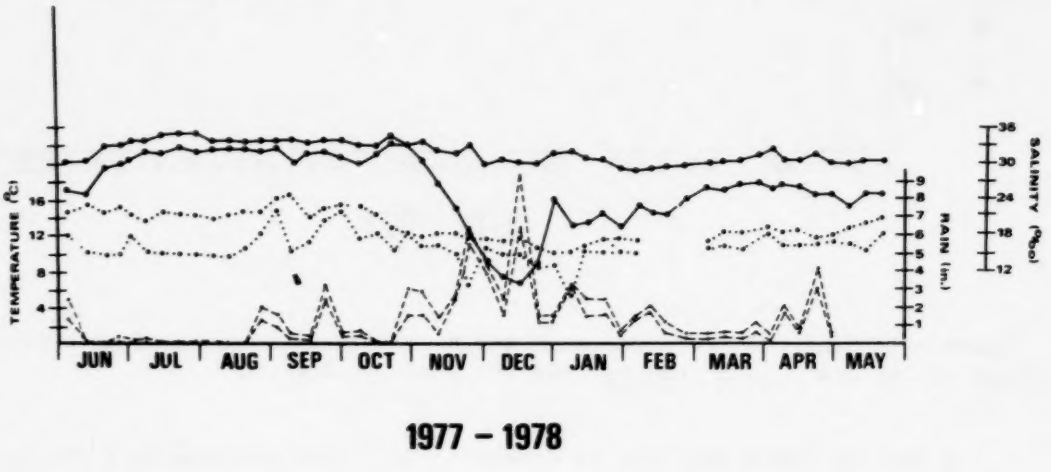


Figure 9. The same information for 1977-78.

The most significant difference between the 2 years was the virtual absence of rain during the winter (Nov.-Feb.) of 1976-77. The amount of rainfall during the winter of 1977-78 was rather typical for the Oregon coast. The influx of freshwater resulted in depressed temperatures and salinity in the estuary during this period.

As a result of the fluctuations in seasonal and environmental parameters during the two years, a two way ANOVA was used to determine if there were differences in seasonal-year correlation for BAP body burdens between 1976-77 and 1977-78. It was determined that there was a significant difference between the two years ($F.01=7.06>F.01\ [5,80]=2.33$). As a result of that statistical test, further analyses were conducted separately for 1976-77 and 1977-78. Figures 10 and 11 show the fluctuations in BAP body burdens during those 2 years. The percent mean body burdens were recalculated using only the data for the respective year.

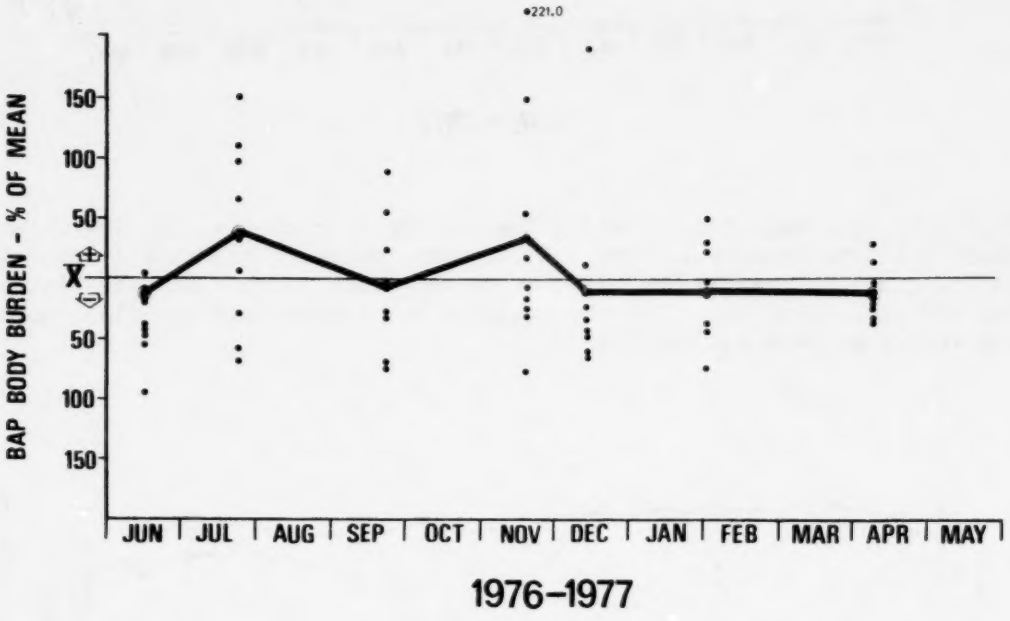


Figure 10. Seasonal differences in BAP body burdens, expressed as a percentage of the mean, in M. edulis from Yaquina Bay, Oregon.

A one way ANOVA was used to determine if there were seasonal effects in BAP body burdens for each of the two years. There were no seasonal effects during 1976-77 ($F=0.26<F.01\ [4,54]=2.54$) but there were seasonal effects during 1977-78 ($F=9.36>F.01\ [5,40]=2.45$). A Student Newman-Keuls Multiple Comparison test was used to identify which "seasons" were significantly

different. Each of the 6 sampling dates (i.e., June, 1977; December, 1977, etc.) was considered to be a season. Only the February, 1978 season differed significantly from the other seasons at the 0.01 level. It should be noted (Fig. 11) that the decline in BAP concentrations during the fall, while not statistically significant, seemed to be a characteristic of all the different mussel populations as evidenced by the tight clustering of data points.

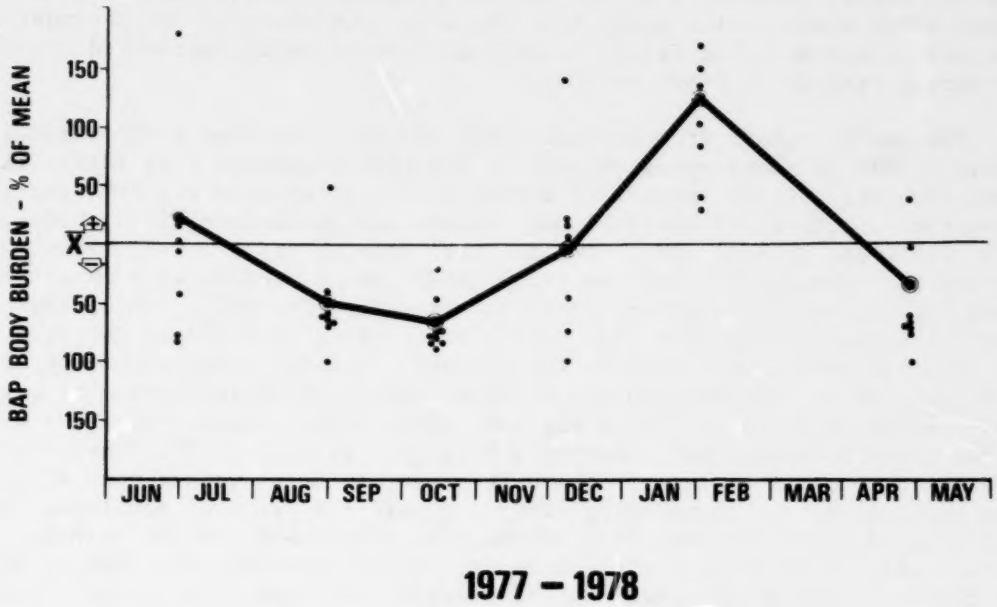


Figure 11. Seasonal differences in BAP body burdens, expressed as a percentage of the mean, in M. edulis from Yaquina Bay, Oregon.

There are several factors that may account for the general seasonal trends of 1977-78. Various mechanisms may be expected to influence the environmental availability of BAP and thus, the amount found in mussels at any particular time. Photo-oxidation of BAP occurs in the presence of sunlight; the greater the amount of sunlight the greater the quantities of BAP oxidized by this process. Thus, the decrease in BAP body burdens during the summer and fall may be related to the fact that August and September are generally months with the most days of sunshine along the Oregon coast. A similar explanation may partially account for the increases in BAP in mussels during the winter months when there are prolonged periods without direct sunlight.

The amount of BAP in the atmosphere would be expected to be higher during the winter since more organic material (oil, coal, gas, wood) is burned to provide heat. An increase of combustion products, including BAP, coupled with an increase in rainfall may result in rainout of atmospheric BAP, thus increasing the environmental levels of BAP in estuaries.

Urban sewage and sewage sledges may contain considerable quantities of PNAH (Harrison et al., 1975). Dunn and Stich (1976) reported that discharges from sewage treatment plants represented a major source of BAP contamination in Vancouver Harbor. Three small towns are situated on Yaquina Bay or its major tributaries, the Yaquina and Elk Rivers. BAP contamination of Yaquina Bay from Elk City (septic tanks) and Newport sewer systems, which empty into the ocean, would be expected to be minimal or nonexistent. Unknown, but probably small, quantities of BAP may be introduced during the winter from Toledo storm sewers which empty into the bay; inundation of the secondary treatment plant may also result in untreated water being introduced into the bay during periods of heavy rainfall.

Freshwater runoff from the watershed may also increase environmental levels of BAP in three ways. First, it has been suggested that forest fires contribute significant amounts of BAP to nearby ecosystems via atmospheric deposition or runoff of residual BAP (Blumer and Youngblood, 1975; Hites, 1976; Clark and McLeod, 1977). While there were no major forest fires in the Yaquina Bay watershed during the study, there was a considerable amount of slash burning in logged areas, particularly during the fall. Thus, the heavy rains of November-December, 1977, may have transported residual BAP from the burned areas of the watershed to the estuary. Second, heavy runoff of freshwater results in the resuspension of sediments in receiving bodies of water. Since sediments serve as a sink for BAP in estuaries, resuspension may also increase environmental BAP. However, Roesijadi et al., (1978) found that PNAH bound to particulate matter was less available for uptake than that fraction released to the surrounding water and Neff (in press a) concluded, after reviewing relevant studies, that sediment-absorbed PNAH are not readily assimilated, at least by benthic animals. Third, unknown, but perhaps substantial, quantities of petroleum hydrocarbons are deposited in urban areas from a variety of sources such as oil heating systems, fallout and the operation of motor vehicles. Rainfall and runoff may flush petroleum materials either into storm drains or directly into rivers or bays (NAS, 1975).

Thus, there are four physical or chemical processes--photo-oxidation, rainout, runoff, resuspension--that may be associated with seasonal fluctuations in the environmental availability of BAP and BAP body burdens in M. edulis. In addition, there may be certain intrinsic biological processes that account for seasonal differences. Figure 12 shows the relationship between BAP body burden and a "gonad index." The gonad index was determined by examining tissue sections of mussels collected from Y1M, Y2M, Y4M, and Y12M (100 mussels/month/site) and measuring their degree of sexual maturity; a value of 0 indicates no gametes in the follicle while 5 is a sexually mature mussel. The apparent relationship may be artifactual since there was no similar relationship in 1976-77. Nevertheless, 1977-78 was a typical year with respect to environmental parameters while 1976-77 was not. If temperature and salinity are involved in synchronizing the spring spawning cycle, then 1977-78 would be expected to be normal with respect to the sexual maturation of mussels. DiSalvo et al. (1975) found that mussels maintained at, or transferred to, a polluted site showed ratios of aromatics in gonadal to somatic tissue of near unity. Contaminated mussels, in a reciprocal transfer, showed a great reduction in this ratio which was attributed to a discharge of gonadal material during spawning, although this was not confirmed. Sexual

maturation involves the activation of lipid synthesizing pathways and perhaps results in an increase in lipid pools containing BAP that is then routed into tissue sinks (gametes). Thus, the spring peaks of BAP may reflect its increasing concentration in gametes; a decrease would then occur after spawning.

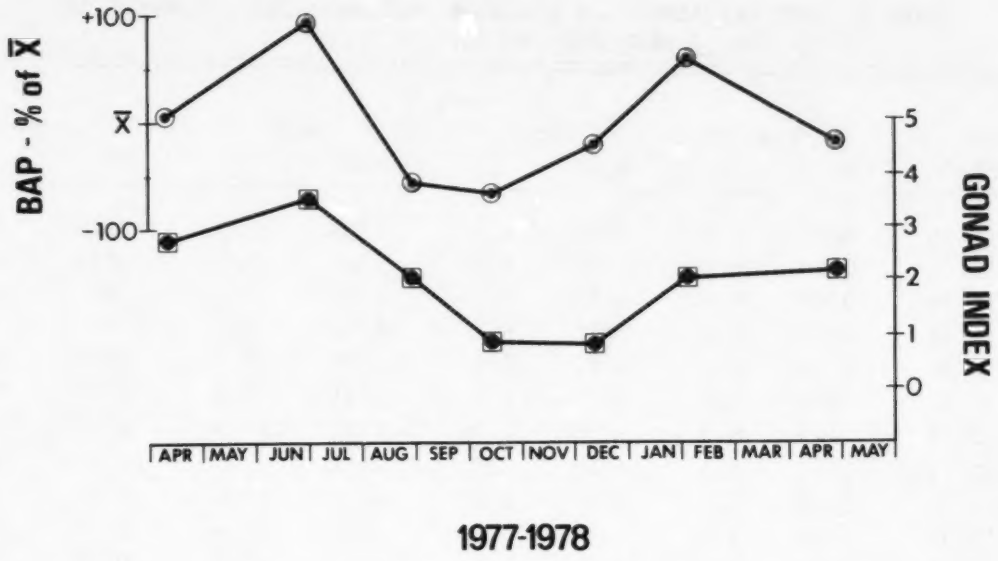


Figure 12. BAP body burdens and the degree of sexual maturity, as measured by the gonad index, in M. edulis from Yaquina Bay, Oregon. Squares represent the mean degree of sexual maturity in mussels from 4 sites; circles indicate the average percentage of the mean BAP body burden in mussels from 4 sites.

Finally, it should be noted that temperature and salinity of the immediate environment affect many physiological functions in marine organisms. These factors also affect solubility, adsorption-desorption kinetics, octanol/water partition coefficients, etc. of PNAH in water (Neff, in press a). In general, PNAH uptake is greater at reduced temperatures while changes in salinity have little or no effect (e.g. Fucik and Neff, 1977). Thus, increased BAP concentrations in mussels during the winter may be related to low water temperatures.

In summary, it is possible that the 1977-78 spring peaks were associated with increases in environmental BAP, intrinsic physiological factors, and/or the effects of endogenous factors on PNAH uptake and incorporation. Dunn and Stich (1976) suggested that seasonal BAP variations in mussels from Vancouber Harbor were attributable to seasonal changes in pollution rather than variations in BAP uptake, retention with water temperature or breeding cycle. Data from the present study neither support nor refute that interpretation.

## CELLULAR PROLIFERATIVE DISORDERS IN M. EDULIS

Table 6 summarizes the data on the prevalence of cellular proliferative disorders in M. edulis from four sites in Yaquina Bay. Y1M and Y12M represent populations of mussels that always had low or undetectable BAP concentrations while Y2M and Y4M were sites where mussels always had high BAP body burdens (Mix et al., in press a).

TABLE 6. THE PREVALENCE OF CELLULAR PROLIFERATIVE DISORDERS IN M. EDULIS FROM YAQUINA BAY

| Date sampled | SITE Y1M No. | Y1M % | Y2M No. | Y2M % | Y4M No. | Y4M % | Y12M No. | Y12M % |
|---|---|---|---|---|---|---|---|---|
| 6/15/76 | 0/10 | 0.0 | 0/10 | 0.0 | 1/10 | 10.0 | 0/10 | 0.0 |
| 7/22/76 | 0/10 | 0.0 | 0/10 | 0.0 | 0/10 | 0.0 | 0/10 | 0.0 |
| 8/22/76 | 0/45 | 0.0 | 0/42 | 0.0 | 6/161 | 3.7 | NS | --- |
| 10/21/76 | 0/194 | 0.0 | NS* | --- | 16/199 | 8.0 | NS | --- |
| 12/16/76 | 0/101 | 0.0 | 12/97 | 12.4 | 13/90 | 14.4 | 0/100 | 0.0 |
| 2/03/77 | 0/100 | 0.0 | 2/46 | 4.4 | 13/51 | 25.5 | 0/97 | 0.0 |
| 4/18/77 | 0/43 | 0.0 | 11/98 | 11.2 | 7/50 | 14.0 | 0/95 | 0.0 |
| 6/29/77 | 1/48 | 2.1 | 4/48 | 8.3 | 0/38 | 0.0 | 0/49 | 0.0 |
| 8/29/77 | 0/48 | 0.0 | 1/48 | 2.1 | 1/49 | 2.0 | 0/46 | 0.0 |
| 10/13/77 | 1/39 | 2.6 | 0/48 | 0.0 | 2/44 | 4.6 | 0/45 | 0.0 |
| 12/08/77 | 0/49 | 0.0 | 3/51 | 5.9 | 7/51 | 13.7 | 0/50 | 0.0 |
| 2/03/78 | 0/48 | 0.0 | 0/50 | 0.0 | 3/49 | 6.1 | 0.48 | 0.0 |
| 4/28/78 | 0/50 | 0.0 | 2/30 | 6.7 | 6/50 | 12.0 | 0/50 | 0.0 |
| TOTAL | 2/785 | 0.25 | 35/578 | 6.1 | 75/852 | 8.8 | 0/600 | 0.0 |

*NS, Not sampled.

Statistical analyses revealed that: (1) there was a site-prevalence correlation; (2) mussels from sites Y2M and Y4M (contaminated sites) had significantly higher prevalences than those from Y1M and Y12M (uncontaminated sites; (3) there was seasonal-prevalence correlation; and (4) the seasonal-prevalence correlation was curvilinear. The seasonal-prevalence correlation was subjected to multiple regression analysis for 2 variables. The data fit a quadratic equation, $\hat{Y}=AX^2+BX+C$ where $\hat{Y}=0.0003X^2-0.1231X+15.7914$ ($\hat{Y}$=prevalence; X=day of the year), and provides an acceptable curvilinear fit to these data ($R^2=0.388$).

Although it is not yet possible to formulate any definitive conclusions, it seems significant that, with two exceptions (n=1,385), no mussels with low body burdens of BAP from the Y1M and Y12M sites had the cellular disorders, whereas substantial numbers (110/1,430) were found in mussels collected from the Y2M and Y4M sites with high body burdens of BAP. However, numerous

inorganic and organic chemicals can induce neoplasia in sensitive species and polluted aquatic environments frequently contain a wide variety of potential carcinogens (Bergel, 1974). A salient question which remains to be answered is whether BAP or any other PNAH can cause neoplasia or the malignant equivalent in bivalve mollusks. The evidence accumulated to date indicates that few, if any, bivalve mollusks possess the enzymes necessary to metabolize BAP to its reactive intermediates (Lee et al., 1972; Neff and Anderson, 1975; Payne, 1977; Vandermeulen and Penrose, 1978; Neff, in press b) although Anderson (1978) detected very low levels of BAP oxidizing enzymes in oysters (C. virginica). If metabolic conversion does not occur, it is necessary to conceive of an alternative metabolic or physiological mechanism by which BAP or other carcinogenic PNAH's could cause neoplastic cell disorders in these organisms, if these cells have, in fact, been transformed.

## ELECTRON MICROSCOPIC ANALYSIS OF THE ATYPICAL, LARGE M. EDULIS CELLS

Studies on the ultrastructure of large cells associated with putative neoplastic disorders of mussels from Yaquina Bay have recently been completed (Mix et al., in press b). Atypical large cells had an average diameter of 15 µm and a nuclear to cytoplasmic ratio of about 1-1.5. Briefly, the large M. edulis cells possess many ultrastructural properties that are characteristic of certain malignant vertebrate cells: large, polymorphic nuclei; large, multiple nucleoli; altered Golgi complexes; mitochondrial inclusions; an absence of cytoplasmic differentiation; ribosomes not associated with a membrane system; pleomorphic inclusions in the cytoplasm; and possible asynchronization in the maturation of nucleus and cytoplasm. Nevertheless, much additional information about their life history is required before any definitive conclusions can be formulated.

It should be noted that many of these properties are also characteristic of normal cells that are engaged in rapid proliferation (Maile, 1972). This last point is particularly important since it suggests another way that high body burdens of BAP, and presumably other PNAH, may be associated with the cellular disorders. It became evident during the analyses that BAP is only one of many PNAH's in the mussels analyzed during this study. Six to ten fluorescent bands were consistently observed in the thin-layer chromatography (TLC) plates which may represent individual PNAH or groups of PNAH's. There was also a direct correlation between the concentration of BAP and the number and size of the fluorescent bands. Therefore, BAP body burdens may represent only a small fraction of the total chemical load associated with PNAH and possibly other anthropogenic environmental insults. Perhaps then, the large cells represent a response to chronic environmental stress with possible functional capabilities associated with detoxification. Such a cellular response may be entirely non-neoplastic, initially non-neoplastic with subsequent neoplastic transformation, or entirely neoplastic.

## PUBLIC HEALTH CONSIDERATIONS

The finding that certain indigenous populations of bivalve mollusks contain substantial body burdens of BAP indicates that a potential public health

problem may exist. However, a review of relevant data indicates that there are numerous problems in estimating the hazard to man from consuming seafood with elevated PNAH content. Gerarde (1960) reported that PNAH's are poorly absorbed by the mammalian GI tract and that 40-97% of BAP was excreted. It is apparently still a matter for debate whether there is any dose-response relationship in man, or a threshold dose below which carcinogens do not induce cancer (WHO, 1972). If there is an effective threshold for oral intake of PNAH in man, the critical question becomes whether this threshold could be exceeded by the PNAH content in contaminated seafood (GESAMP, 1977). Except for the possible association between heavily smoked fish and a high incidence of stomach cancer in Iceland and Slovenia (Wynder et al., 1963), no epidemiological studies have linked GI cancers in man with the ingestion of marine fish or shellfish. The presence of ppb concentrations of BAP and other PNAH in a number of common foods such as fresh vegetables, meat, fish and poultry has led to the suggestion that trace amounts of carcinogenic PNAH do not constitute a health hazard (Friedman, 1974); obviously, this opinion is not universally accepted by all scientists. Clearly, a great deal of additional information is required to accurately assess the risk of consuming seafood contaminated with varying levels of PNAH's.

There are several other questions to consider before it can be determined whether or not a human health hazard exists.

Those questions, with partial answers, are listed below.

1. Is the shellfish species economically important (i.e., is it exploited as a food source)? M. edulis, the species with the greatest body burdens, is not heavily exploited by Oregon shellfishermen. M. arenaria is subjected to moderate pressure while T. capax, S. giganteus, and C. nuttalli are heavily exploited. M. arenaria from Coos Bay was the only clam species with significant concentrations of BAP. Oysters (C. gigas) did not have significant body burdens of BAP.

2. Are the heavily contaminated species found in areas accessible to and harvested by shellfishermen? Heavily contaminated M. edulis from Yaquina Bay and M. arenaria from Coos Bay are accessible to the public; however, the former species is not generally utilized while the latter species is not subjected to harvesting pressure at the highly contaminated sites (C4S and C19S). Lightly contaminated clams from T6G, T7G, T10S, Y15G, Y16G, Y17G, C5S, C7S, C9G, C10G, C11G, C12G, C13G, C16B, and C17B are all accessible and subjected to moderate to heavy digging pressure throughout the year as low tides permit.

3. What is the tissue storage time for BAP and other PNAH? Relatively little information is available concerning the extent to which BAP and other PNAH are accumulated and eliminated by shellfish. Research on rates of uptake and elimination (RUE) have generally been conducted in the laboratory (Lee, et al., 1972; Stegeman and Teal, 1973; DiSalvo, et al., 1975; Neff, et al., 1976; Roesijadi, et al., 1978); few field studies have been conducted Dunn and Stich, 1976). Results from the various studies seem to suggest that BAP may be stored in two compartments by clams, oysters, and mussels. Most BAP may initially be contained within a fluid compartment where environmental equilibration would be expected to occur rapidly. The rate of loss from this

compartment would be quite rapid (hours to days) and many laboratory studies have probably analyzed BAP depuration from this depot. Lesser amounts of BAP appear to be stored in a tissue compartment. Intrinsic lipid/water partition coefficients favor the rapid transfer from the aqueous phase into lipophilic compartments (e.g. membranes, macromolecules) (Neely et al., 1974). The precise storage sites of accumulated PNAH are not known but are generally thought to include the lipid stores of the tissues (Stegeman and Teal, 1973; Vandermeulen and Penrose, 1978). However, both environmental equilibration and the rate of loss from tissues would take considerable time (weeks to months), so storage in this compartment would be expected to be associated with any chronic effects of environmental contamination. Few studies have addressed all these aspects of PNAH contamination of shellfish. Generally, the available results indicate that BAP will be relatively persistent in tissues of exposed bivalves (Roesijadi, et al., 1978).

4. Is BAP, and other PNAH, metabolically altered by the bivalve species? As indicated previously, the evidence accumulated to date indicates that bivalve mollusks do not possess the complicated microsomal enzyme systems necessary for metabolic activation of PNAH. Thus, the absence of such enzyme activity may result in the retention of unaltered PNAH in shellfish. Since humans possess the enzymes necessary to convert unaltered PNAH to highly mutagenic or carcinogenic derivatives, retention of unaltered PNAH, if present in significant quantities, could pose a public health problem.

5. What is the total body burden of all PNAH and possibly other chemical carcinogens, in shellfish contaminated with BAP? As indicated previously, BAP seems to serve as an indicator PNAH. Thus, shellfish that are heavily contaminated with BAP will likely have high concentrations of other PNAH. A full understanding of the quantitative aspects of this relationship will be necessary to evaluate the potential public health problem associated with the consumption of contaminated shellfish.

6. What are the sources of environmental BAP and PNAH in contaminated marine organisms? With respect to the current study, potential sources include, but are not limited to: small fuel or oil spills, creosote, fish processing factories, marinas, large ships, pleasure craft, wood products industries, sewage treatment plants, runoff through storm sewage, runoff from logged areas where slash burning has occurred (BAP produced from combustion) and rainout of atmospheric BAP produced from combustion of organic material.

There is no clear risk to users of the shellfish resources of Oregon at the present time. Future studies should be directed toward obtaining more completed answers to these questions in order to assess the potential public health hazard.

## REFERENCES


1. Alderman, D. J., P. VanBanning and A. Perez-Colomer. Two European Oyster (Ostrea edulis) Mortalities Associated with an Abnormal Hemocyte Condition. Aquaculture, 10:335-340, 1977.

2. Anderson, R. S. Benzo(a)pyrene Metabolism in the American Oyster, Crassostrea virginica. EPA-60013-78-009. U.S. Environmental Protection Agency, Gulf Breeze, Florida, 1978. 26 pp.

3. Barry, M. and P. P. Yevich. The Ecological, Chemical and Histopathological Evaluation of an Oil Spill Site. Part III. Mar. Poll. Bull., 6: 171-173, 1975.

4. Bergel, F. Carcinogenic Hazards in Natural and Manmade Environments. Proc. Roy. Soc. London. Ser. B, 185:165-181, 1974.

5. Blumer, M. and W. W. Youngblood. Polycyclic Aromatic Hydrocarbons in Soils and Recent Sediments. Science, 188:53-55, 1975.

6. Brown, R. A. and F. T. Weiss. Fate and Effect of Polynuclear Aromatic Hydrocarbons in the Aquatic Environment. Publication No. 4297. American Petroleum Institute, 1978. 23 pp.

7. Brown, R. S., R. E. Wolke, S. B. Saila and C. W. Brown. Prevalence of Neoplasia in 10 New England Populations of the Soft-Shell Clam (Mya arenaria). Ann. N. Y. Acad. Sci., 298:522-534, 1977.

8. Clark, R. C., Jr., and W. D. McLeod, Jr. Inputs, Transport Mechanisms, and Observed Concentrations of Petroleum in the Marine Environment. In: Effects of Petroleum on Arctic and Subarctic Marine Environments and Organisms, Vol. I., D. C. Malins, ed. Academic Press, N. Y., 1977. pp. 91-223.

9. Couch, J. A. Aquatic Species as Possible Indicators of Environmental Carcinogens. NAS Symposium Volume: Pathobiology of Environmental Pollutants in Animal Models and Wildlife as Monitors, In Press.

10. DiSalvo, L. H., M. E. Guard and L. Hunter. Tissue Hydrocarbon Burden of Mussels as a Potential Monitor of Environmental Hydrocarbon Insult. Environ. Sci. Technol., 9:247-251, 1975.

11. Dunn, B. P. Techniques for Determination of Benzo(a)pyrene in Marine Organisms and Sediments. Environ. Sci. Technol., 10:1018-1021, 1976.

12. Dunn, B. P. and H. F. Stich. The Use of Mussels in Estimating Benzo-(a)pyrene Contamination of the Marine Environment. Proc. Soc. Exp. Biol. Med., 150:49-51, 1975.

13. Dunn, B. P. and H. F. Stich. Monitoring Procedure for Chemical Carcinogens in Coastal Waters. J. Fish. Res. Board Can., 33:2040-2046, 1976.

14. Dunn, B. P. and D. R. Young. Baseline Levels of Benzo(a)pyrene in Southern California Mussels. Mar. Poll. Bull., 7:231-233, 1976.

15. Feldman, M. H. Petroleum Weathering: Same Pathways, Fate and Disposition on Marine Waters. EPA 660/3-73-013, U.S. Environmental Protection Agency, 1973. 22 pp.

16. Friedman, L. Assessment of the Carcinogenicity and Mutagenicity of Chemicals. Geneva Technical Report Series No. 546, World Health Organization, Geneva, Switzerland, 1974. pp. 14-19.

17. Fucik, K. W. and J. M. Neff. Effects of Temperature and Salinity on Naphthalenes Uptake in the Temperate Clam Rangia cuneata and the Boreal Clam Prototheca staminea. In: Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems, D. A. Wolfe, ed. Pergamon Press, Oxford, 1977. pp. 305-316.

18. Gerarde, H. W. Toxicology and Biochemistry of Aromatic Hydrocarbons. Elsevier, London, 1960.

19. GESAMP. IMCO/FAO/WMO/WHO/IAEA/UN. Joint Group of Experts on the Scientific Aspects of Marine Pollution. Impact of Oil on the Marine Environment. Rep. Study No. 6. FAO, 1977. 250 pp.

20. Goldberg, E. D. The Mussel Watch. A First Step in Global Marine Monitoring. Mar. Poll. Bull. 6:111, 1975.

21. Harrison, R. M., R. Perry and R. A. Wellings. Polynuclear Aromatic Hydrocarbons in Raw, Potable and Waste Waters. Water Research, 9: 331-346, 1975.

22. Higgenson, J. The Role of Geographical Pathology in Environmental Carcinogenesis. In: Environment and Cancer. The Williams and Williams Co., 1971. pp. 68-69.

23. Hites, R. A. Sources of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment. In: Proceedings AIBS Symposium on Sources, Effects and Sinks of Hydrocarbons in the Aquatic Environment, 1976. pp. 325-331.

24. Kraybill, H. F. Distribution of Chemical Carcinogens in Aquatic Environments. Prog. Exptl. Tumor Res., 20:3-34, 1976.

25. Kraybill, H. F. Introductory Remarks: Overview on Aquatic Pollutants and their Biologic Effects. Ann. N. Y. Acad. Sci., 298:1, 1977.

26. Lee, R. F. Accumulation and Turnover of Petroleum Hydrocarbons in Marine Organisms. In: Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms, D. A. Wolfe, ed. Pergamon Press, Oxford, 1977. pp. 60-70.

27. Lee, R. F., R. Sauerheber and A. A. Benson. Petroleum Hydrocarbons: Uptake and Discharge by the Marine Mussel, Mytilus edulis. Science, 177:344-346, 1972.

28. Lowe, D. M. and M. N. Moore. Cytology and Quantitative Cytochemistry of a Proliferative Atypical Hemocytic Condition in Mytilus edulis (Bivalvia, Mollusca). J. Natl. Cancer Inst., 60:1455-1459, 1978.

29. Maile, J. B. Laboratory Medicine: Hematology, Fourth Edit. The C. V. Mosby Co., St. Louis, Mo., 1972. 982 pp.

30. Mix, M. C., R. T. Riley, K. I. King, S. R. Trenholm, and R. L. Schaffer. Chemical Carcinogens in the Marine Environment. Benzo(a)pyrene in Economically Important Bivalve Mollusks from Oregon Estuaries. In: Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems, D. A. Wolfe, ed., Pergamon Press, Oxford, 1977a. pp. 421-431.

31. Mix, M. C., J. Pribble, R. T. Riley, and S. P. Tomasovic. Neoplastic Disease in Marine Animals from Oregon Estuaries with Emphasis on Research on Proliferative Disorders in Yaquina Bay Oysters. Ann. N. Y. Acad. Sci., 298:356-373, 1977b.

32. Mix, M. C., S. R. Trenholm and K. I. King. Benzo(a)pyrene Body Burdens and the Prevalence of Cellular Proliferative Disorders in Mussels, Mytilus edulis, from Yaquina Bay, Oregon. NAS Symposium Volume: Pathobiology of Environmental Pollutants - Animal Models and Wildlife as Monitors, In Press a.

33. Mix, M. C., J. W. Hawkes and A. K. Sparks. Observations on the Ultrastructure of Large Cells Associated with Putative Neoplastic Disorders of Mussels, Mytilus edulis, from Yaquina Bay, Oregon. J. Invertebr. Pathol., In Press b.

34. National Academy of Sciences. Petroleum in the Marine Environment. Washington D. C., 1975. 107 pp.

35. Neely, W. B., D. R. Branson, and G. E. Blau. Partition Coefficient to Measure Bioconcentration Potential of Organic Chemicals in Fish. Environ. Sci. Technol., 8:1113-1115, 1974.

36. Neff, J. M. Accumulation and Release of Polycyclic Aromatic Hydrocarbons from Water, Food, and Sediment by Marine Animals. In: Symposium on Carcinogenic Polynuclear Aromatic Hydrocarbons in the Marine Environment. Pensacola, Florida, In Press a.

37. Neff, J. M. Polycyclic Aromatic Hydrocarbons in the Aquatic Environment and Cancer Risk to Aquatic Organisms and Man. In: Symposium on Carcinogenic Polynuclear Aromatic Hydrocarbons in the Marine Environment. Pensacola, Florida, In Press b.

38. Neff, J. M. and J. W. Anderson. Accumulation, Release and Body Distribution of Benzopyrene $^{14}C$ in the Clam Rangia cuneata. Proc. Joint Conference on Prevention and Control of Oil Spills, 1975. pp. 469-471.

39. Neff, J. M., B. A. Cox, D. Dixit and J. W. Anderson. Accumulation and Release of Petroleum - Derived Aromatic Hydrocarbons by Four Species of Marine Animals. Mar. Biol., 38:279-289, 1976.

40. Payne, J. F. Mixed Function Oxidases in Marine Organisms in Relation to Petroleum Hydrocarbon Metabolism and Detection. Mar. Poll. Bull., 8: 112-116, 1977.

41. Percy, K. L., C. Sutterlin, D. A. Bella and P. C. Klingeman. Descriptions and Information Sources for Oregon Estuaries. O. S. U. Sea Grant Program, Corvallis, Oregon, 1974.

42. Roesijadi, G., J. W. Anderson and J. W. Blaylock. Uptake of Hydrocarbons from Marine Sediments Contaminated with Prudhoe Bay Crude Oil: Influence of Feeding Type of Test Species and Availability of Polycyclic Aromatic Hydrocarbons. J. Fish. Res. Board Can., 35:608-614, 1978.

43. Spurr, A. R. A Low Viscosity Epoxy Resin Embedding Medium for Electron Microscopy. J. Ultrastruct. Res., 26:31-43, 1969.

44. Stegeman, J. J. and J. T. Teal. Accumulation, Release and Retention of Petroleum Hydrocarbons by the Oyster Crassostrea virginica. Mar. Biol., 22:37-44, 1973.

45. Vandermeulen, J. H. and W. R. Penrose. Absence of Aryl Hydrocarbon Hydroxylase (AHH) in Three Marine Bivalves. J. Fish. Res. Board Can., 35:643-647, 1978.

46. WHO. Health Hazards of the Human Environment. World Health Organization, Geneva, 1972. 188 pp.

47. Wynder, E. L., J. Kmet, N. Dungal and M. Segi. An Epidemiological Investigation of Gastric Cancer. Cancer, 16:1461-1496, 1963.

48. Yevich, P. P. and C. A. Barszcz. Neoplasia in Soft-Shell Clams (Mya arenaria) Collected From Oil-Impacted Sites. Ann. N. Y. Acad. Sci., 298:409-426, 1977.

49. Zobell, C. E. Sources and Biodegradation of Carcinogenic Hydrocarbons. In: Proceedings of the Joint Conference on Prevention and Control of Oil Spills, 1971. pp. 441-451.